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Control of Singlet-Precursor Radical Pair Lifetime by Manipulation of Spin-Selective Reaction Pathways

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Radicals produced by illumination or ionizing radiation are often produced in pairs, which quickly decay by recombination or by diffusion and subsequent reaction. For studying reaction pathways and optimizing the yield of radical pair products it is desirable to minimize the probability of charge recombination. We present here a method based on simple radical pair theory to effectively prolong the lifetime of radical pairs that are formed from an excited singlet state by manipulating the population of energetically isolated radical pair triplet levels. Two experimental schemes are discussed and demonstrated on the secondary radical pair of photosynthetic reaction centers. For this system we have achieved an increase in radical pair lifetime of 1−2 orders of magnitude at low temperatures (7 K).

Introduction

It has been know for quite some time that the application of a magnetic field may change the yield and/or lifetime of radical pair states and products. One of the early pioneers of the field is Saburo Nagakura.[1] Most theories explaining magnetic field effects on chemical reactions have been developed using the concept of the so-called correlated radical pair (see refs 2−4 for reviews on magnetic field effects). This concept has proved fruitful in explaining the effect of an external magnetic field on molecular triplet yield, chemically induced dynamic electron spin polarization (CIDEP), chemically induced dynamic nuclear polarization (CIDNP), and reaction yield detected magnetic resonance (RYDMAR).

For studying reaction pathways or radical pair products it is desirable to significantly decrease the probability of charge recombination. Although changes in lifetime are observed in the above mentioned experiments, these effects are either very small or only applicable to radical pairs that are formed from an electronic triplet state.[5−8] In this work we will show in a simple application of the correlated radical pair theory of magnetic field effects that when forward reactions are (much) slower than recombination, it is possible to prolong the lifetime of a considerable fraction of radical pairs that are formed from an excited singlet state by more than 1 order of magnitude. The method is based on populating energetically isolated spin states of the radical pair in a magnetic field. These isolated spin states can often decay only by spin−lattice relaxation to nonisolated states, followed by charge recombination. Often the spin−lattice relaxation time is orders of magnitude larger then the charge-recombination time, thus providing a tool to prolong the radical pair lifetime quite effectively. We will first briefly review the theory of correlated radical pairs and then demonstrate two efficient methods for lifetime prolongation on radical pairs that initially have no net spin angular momentum.

Theory

Photochemical and radiolytic chemical reactions often proceed from a radical pair created through charge separation by electron or hole transfer. In many photochemical reactions the radical pair is created by electron transfer from the excited singlet state of the donor D to the acceptor A; DA + $h\nu \rightarrow$ D*A $\rightarrow$ D^+A^-. The two charged products that form the radical pair are both doublet states; $s = 1/2$. The total spin function ($S = s_1 + s_2$) of the radical pair thus can have a total spin angular momentum $S = 0$ or $S = 1$; that is, it can be in an electronic singlet or triplet state. These pure singlet and triplet functions, however, are in general non-eigenfunctions of the radical pair spin Hamiltonian. In the next two sections we will discuss the radical pair eigenfunctions in the two limiting cases of zero and high magnetic field.

The Radical Pair in a (High) Magnetic Field. For calculating the eigenfunctions of the radical pair in a magnetic field it is convenient to use the following spin functions as a basis set:

$$\begin{aligned} |S\rangle &= |0,0\rangle \\ |T_+\rangle &= |1,+1\rangle \\ |T_0\rangle &= |1,0\rangle \\ |T_-\rangle &= |1,-1\rangle \end{aligned} \tag{1}$$

where for the state $|i,j\rangle$ i and j represent the quantum numbers for the total spin angular momentum, S, and the z-projection of S, respectively.

The spin Hamiltonian of the radical pair in a magnetic field is given by

$$H_{rp} = H^{D}_{Zeeman} + H^{A}_{Zeeman} + H^{DA}_{dipolar} + H^{DA}_{exchange} \tag{2}$$

and includes the Zeeman interaction of both spins with the magnetic field and the mutual dipolar and exchange interactions. The following functions are the eigenstates of the radical pair

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Hamiltonian when the Zeeman interaction is large compared to the other terms in the spin Hamiltonian:[9−11]

$$\begin{aligned}|1\rangle &= |\mathrm{T}_+\rangle\\ |2\rangle &= +\cos(\phi)|\mathrm{S}\rangle + \sin(\phi)|\mathrm{T}_0\rangle\\ |3\rangle &= -\sin(\phi)|\mathrm{S}\rangle + \cos(\phi)\,|\mathrm{T}_0\rangle\\ |4\rangle &= |\mathrm{T}_-\rangle\end{aligned} \tag{3}$$

with

$$2\phi = \arctan\left(\frac{2Q}{2J + D(\cos^2\Theta - 1/3)}\right) \tag{4}$$

in which J is the exchange interaction, D is the axial dipolar interaction parameter, Θ is the angle between the dipolar z-axis and the magnetic field direction, and

$$Q = {}^1\!/_2(\omega_\mathrm{D} - \omega_\mathrm{A}) \tag{5}$$

with $\omega_\mathrm{D} - \omega_\mathrm{A}$ the difference between the Larmor frequencies of the donor D and acceptor A, induced by local differences in g-factor and hyperfine interactions.

When the parent of the radical pair is an electronic singlet state, the total spin angular momentum of the radical pair is zero initially. Because this state is a non-eigenstate of the radical pair spin Hamiltonian (eq 2), the wave function will start to oscillate between the singlet state $|\mathrm{S}\rangle$ and the triplet state $|\mathrm{T}_0\rangle$. When these oscillations are damped, states $|2\rangle$ and $|3\rangle$ are populated proportional to their singlet character given by $\sin^2\phi$ and $\cos^2\phi$, respectively. The states $|1\rangle$ and $|4\rangle$ are not populated except for spin−lattice relaxation, which was not taken into account in the Hamiltonian of eq 2. The spin−lattice relaxation is usually much slower then radical pair recombination; hence almost no radical pair states $|1\rangle$ and $|4\rangle$ are formed and the radical pair decays through charge recombination from states $|2\rangle$ and $|3\rangle$ to either the singlet ground state or the molecular triplet state ^{3}D or ^{3}A. In the following we will assume that forward reactions from the radical pair are blocked, or slow with respect to recombination, and that the molecular triplet states are energetically not accessible, so that radical pair decay will only proceed to the singlet ground state. This situation often occurs when radical pairs are stabilized by electron or hole transport in a chain of donor−acceptor molecules.

The Radical Pair in Zero Field. In zero external magnetic field the functions of eq 3 are not convenient since there is no clear direction of quantization for the spin angular momentum. A more convenient set of basis functions then is[12]

$$\begin{aligned}&|\mathrm{S}\rangle\\ |\mathrm{T}_x\rangle &= (1/\sqrt{2})(|\mathrm{T}_-\rangle - |\mathrm{T}_+\rangle)\\ |\mathrm{T}_y\rangle &= (i/\sqrt{2})(|\mathrm{T}_-\rangle + |\mathrm{T}_+\rangle)\\ |\mathrm{T}_z\rangle &= |\mathrm{T}_0\rangle\end{aligned} \tag{6}$$

When the dipolar and exchange interactions are small, these four functions are quasi-degenerate and are therefore strongly mixed when Q is comparable to D and J. The four radical pair eigenstates are composed of about equal amounts of all four basis functions from eq 6, so that the four eigenstates are populated with almost equal probability after the damping of the oscillations.

Lifetime Control. The two methods we discuss here for prolonging radical pair lifetime are both based on populating the high-field eigenstates $|1\rangle$ and $|4\rangle$. When a radical pair is in one of these two states, the only way for charge recombination is through spin−lattice relaxation back to one of the singlet-character-containing states $|2\rangle$ or $|3\rangle$, followed by normal charge recombination. Two independent experimental schemes to achieve population of the isolated levels are discussed and compared below.

1. Microwave Irradiation. When the radical pair is created in high field, initially only the states $|2\rangle$ and $|3\rangle$ are populated. Under normal conditions the radical pair recombines before any relaxation process can occur. When, however, directly after the formation of the radical pair, a pulse of microwaves is applied that is resonant between the radical pair states $|2\rangle$,$|3\rangle$ and $|1\rangle$,$|4\rangle$ from eq 3, we transfer population from states $|2\rangle$ and $|3\rangle$ to states $|1\rangle$ and $|4\rangle$. After the pulse the $|1\rangle$ and $|4\rangle$ populations are again isolated and, as argued above, can only decay via spin−lattice relaxation to $|2\rangle$ and $|3\rangle$. As a result, radical pair decay is slowed down to the spin−lattice relaxation time for the fraction of radical pairs that are in states $|1\rangle$ and $|4\rangle$. The fraction of $|2\rangle$ and $|3\rangle$ population actually transferred by the microwave pulse to $|1\rangle$ and $|4\rangle$ depends on the triplet character, which determines the transition probability, and the singlet character, which determines the population. When the splitting of $|2\rangle$ and $|3\rangle$ is smaller than or comparable to the microwave field B_1, the microwave pulse will connect level $|2\rangle$ with $|1\rangle$ and $|4\rangle$, and similarly for level $|3\rangle$. The populations of states $|1\rangle$ and $|4\rangle$ immediately after a microwave pulse of length τ are both[13,14]

$$ {}^1\!/_8 \sin^2(2\phi)\sin^2(\gamma B_1 \tau) \tag{7}$$

where ϕ is defined by eq 4, γ is the gyromagnetic ratio, and B_1 is the strength of the microwave field.

The general idea of populating T_+ and T_- radical pair levels by microwave irradiation was suggested by Salikhov and Molin[15] and experimentally demonstrated by Dzuba *et al.*[16]

2. Switching of Magnetic Fields. The second method is based on the fact that the radical pair levels are (almost) degenerate at zero external field. When we create the radical pair in zero field and switch on a magnetic field shortly after the creation of the pair but well within its lifetime, we would expect to trap part of the population in the long-living states $|1\rangle$ and $|4\rangle$. When the zero-field levels are quasi-degenerate, i.e. for a radical pair with small dipolar and exchange interaction, and when a magnetic field that is large compared to the dipolar and exchange interactions is switched on fast compared to the radical pair lifetime, we expect to trap 50% of the initial radical pair population in states $|1\rangle$ and $|4\rangle$.

When the field is not large enough, the radical pair eigenfunctions are a linear combination of the completely mixed zero-field functions and the completely isolated high-field functions; hence the levels $|1\rangle$ and $|4\rangle$ will be mixed to some extent with states $|2\rangle$ and $|3\rangle$. In that case we expect radical pair decay to be intermediate between the decay without magnetic field and the decay for a large magnetic field.

Materials and Methods

As a test sample for our experiments we used photosynthetic reaction centers (RCs) prepared following ref 17, from the purple bacterium *Rhodobacter sphaeroides*. In the RC charge separation occurs after photoexcitation of the primary electron donor (a bacteriochlorophyll dimer) via the primary acceptor to the secondary acceptor, a quinone molecule.[17−20] Subsequent

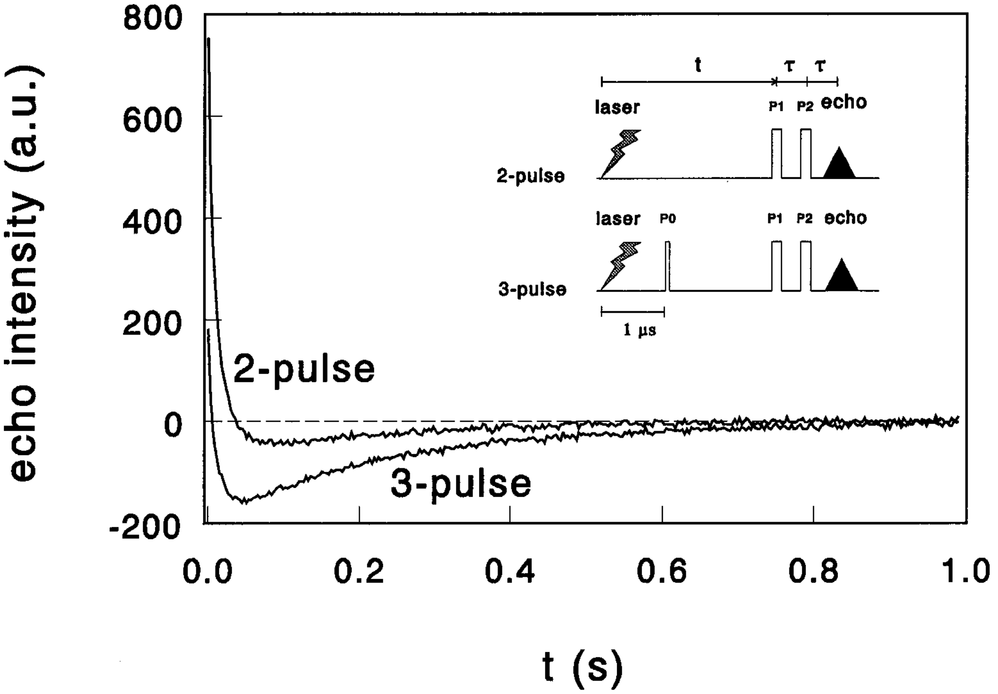

Figure 1. Amplitude of the Hahn echo as a function of time after the laser flash in photosynthetic reaction centers of the purple bacterium *Rb. sphaeroides* strain R26 in which the native paramagnetic iron center was replaced by a diamagnetic Zn^{2+} ion. The wavelength of the laser light was 890 nm with a 5 ns pulse duration (300 mJ/pulse). The Hahn echo sequence consisted of two microwave pulses of 16 ns duration, resonant between the triplet levels of the radical pair (magnetic field ca. 300 mT). The interval between the two pulses was 200 ns. In the three-pulse experiment, an additional pulse is applied of 8 ns duration about 1 μs after the laser flash. The pulse schemes used are depicted in the inset. Adapted from ref 16.

steps are blocked at cryogenic temperatures, and the radical pair recombines with a biexponential decay (10 ms (60%) and 40 ms) to the ground state.[21-24] The radical pair decay kinetics are nearly temperature independent from 1 to 80 K. The energy of the secondary radical pair state is much lower than that of ^{3}D or ^{3}A so that recombination is exclusively to the singlet ground state DA even at room temperature. The exchange interaction between D and A is a few microteslas and the dipolar coupling about 0.1 mT, determined from simulations of electron spin polarization spectra (Van den Brink *et al.*[25]).

Native RCs contain a nonheme iron center, which considerably enhances spin−lattice relaxation through the coupling of the paramagnetic Fe^{2+} ion to the electron spin residing on the acceptor quinone. For our experiments we therefore used RCs that contain a diamagnetic Zn^{2+} ion instead of the iron. These RCs were either chemically modified or genetically manipulated (HisM266Cys mutant). The Zn-containing RCs show the same electron transfer kinetics but have a substantially slower spin−lattice relaxation. The RCs were prepared according to ref 17; chemical Zn-reconstitution was done following ref 26. For the pulsed microwave experiments a home-built electron spin echo spectrometer was used, as described in ref 27. The sample was cooled by an Oxford helium-flow cryostat, and photoexcitation of the primary electron donor was done with a 5 ns pulse at 890 nm from a Continuum Surelite I laser pumping an optical parametric oscillator (output ~300 mJ/pulse).

The switched magnetic field experiments were done with a setup of local design. The sample was mounted between a pair of water-cooled Helmholtz coils fed by two Oltronic B32-20R power supplies operated in series. The power supplies were switched by a field effect transistor, the magnetic field had a 9 ms risetime (1/*e*), and the maximum field was about 80 mT. Photoexcitation of the primary electron donor was done using a xenon flash lamp (~100 mJ/flash) focused on the sample at a right angle to the probe light. The (transient) transmission of the sample was monitored at the wavelength of maximum absorption of the donor singlet ground state with a simple single-beam spectrometer and recorded on a digital averaging oscilloscope. The steady-state transmission was subtracted from the transient with a Textronics AM 502 differential amplifier, which also functioned as a high-frequency filter above 3 kHz.

Results and Discussion

Microwave Pulses. When the amplitude of the Hahn echo is monitored after laser excitation, the decay of the radical pair by charge recombination is clearly visible (Figure 1, two-pulse trace). In this case all radical pair population is residing in levels $|2\rangle$ and $|3\rangle$, and the EPR spectrum is indicated by the stick spectrum in Figure 2, box A.[10,11,28]

When an additional pulse of microwaves is applied prior to the detecting Hahn echo sequence but after the radical-producing laser pulse, a slow-decaying negative tail appears in the decay trace (Figure 1, three-pulse trace). The first part of the decay is thought to arise from the radical pair still in states $|2\rangle$ or $|3\rangle$ after the first microwave pulse. These radical pairs recombine in the normal way. Part of the population is transferred, however, to states $|1\rangle$ and $|4\rangle$, so after states $|2\rangle$ and $|3\rangle$ have recombined we are left with an inverted EPR spectrum, which decays through slow spin−lattice relaxation processes. The inverted spectrum is shown in Figure 2, box B.

One must keep in mind that the stick spectra of Figure 2 need to be dressed by a line shape. For small values of *D* and *J*, appreciable overlap of emissive and absorptive lines occurs, and the resulting EPR intensity is only a few percent of the individual line intensity. For this reason the measurement is very sensitive to small changes in population differences between the radical pair states. This explains the small negative part visible in the two-pulse trace of Figure 1 at late times. It is caused by a small portion of radical pairs that undergo spin−lattice relaxation to one of the long-living levels during their

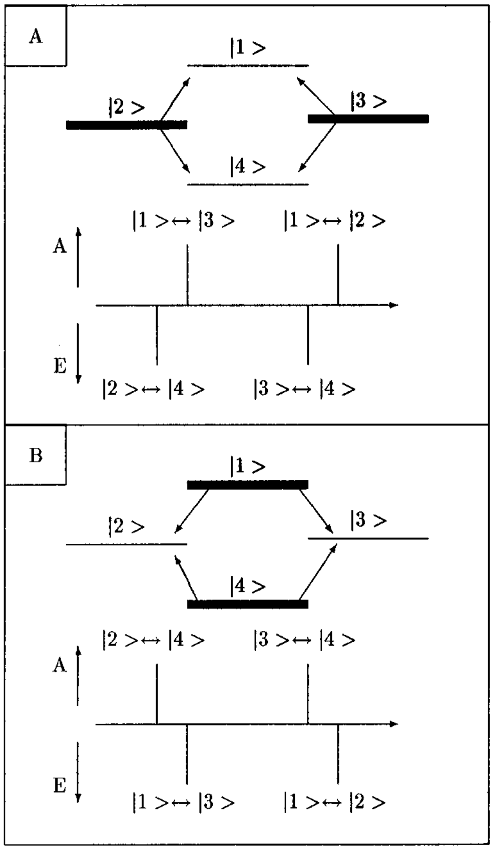

Figure 2. Energy levels, relative populations, and stick EPR spectra for the spin-correlated radical pair. In part A the normal situation is displayed for a radical pair created in high field when only states $|2\rangle$ and $|3\rangle$ are populated (indicated by the thick line). In part B the situation is indicated after a pulse of resonant microwaves is applied as in our experiment, resulting in an inverted polarization of the EPR spectrum after the population of levels $|2\rangle$ and $|3\rangle$ have disappeared through charge recombination.

lifetime. Because of the above-described "cancellation" effect, this part of the population gives a disproportionately large signal.[28]

It was confirmed (data not shown) by measuring the ESEEM "fingerprint"[29] of the fast positive and the slow negative part of the signal that both indeed arise from the same radical species.

Switched Magnetic Field. In Figure 3 the decay after a flash with the xenon flash lamp is plotted for different magnetic fields switched on directly after the flash. The expected increase in lifetime is clearly visible in the curves for the higher fields.

In Figure 4 the decay is plotted at different temperatures. The slow components become faster with increasing temperature, indicating a strong temperature dependence of the spin–lattice relaxation time.

The "field on" decays could not be fitted with the sum of a limited number of exponentials. The fits were unstable, depending on the starting values and the baseline chosen.

Because nonexponential behavior of the decay is expected to occur mainly during the first tens of milliseconds when the field is still rising, we fitted the curves using only the data for $t > 50$ ms. Now three exponentials gave reasonable fits with residuals less than 1%. In all fits we find one short-living component of 30–40 ms, which we ascribe to the radical pairs in states $|2\rangle$ and $|3\rangle$. After the field has been switched on, they decay through the normal recombination channel. We think that the two longer time constants correspond to the radical pairs that are in states $|1\rangle$ and $|4\rangle$ after the field has reached maximum amplitude; they probably represent a distribution of lifetimes.

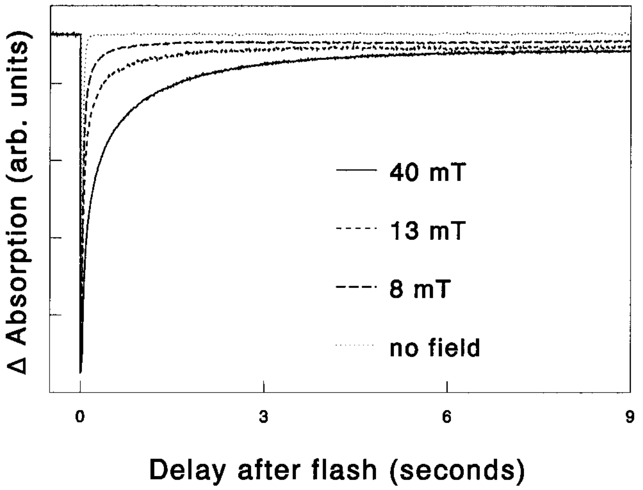

Figure 3. Decay of the ground state bleaching of the primary donor of reaction centers of the bacterium *Rb. sphaeroides* (mutant HisM266Cys) monitored at 890 nm, the absorbance maximum of the donor singlet ground state, with a magnetic field switched on and triggered simultaneously with the xenon flash. The temperature was 23 K; the rise time of the field was 9 ms. All curves are the average of 10–30 single traces. The repetition rate of the experiment was 2 shots/min to avoid excess heating of the Helmholtz coils.

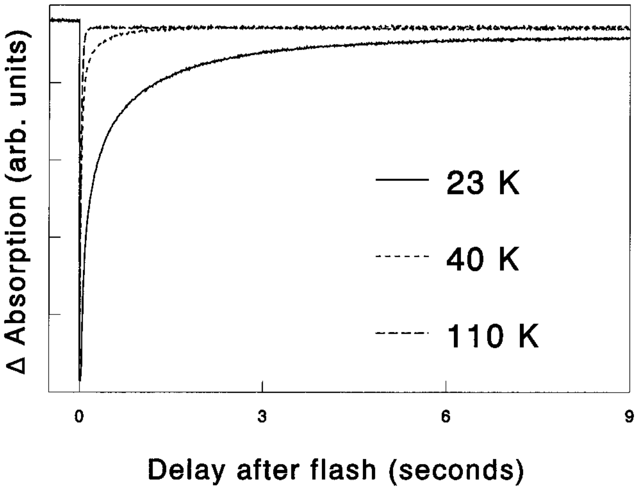

Figure 4. Decay as in Figure 3 for different temperatures. The field was 40 mT with a rise time of 9 ms and was triggered simultaneously with the xenon flash. All curves are the average of 10–30 single traces.

We extrapolated the three exponentials to $t = 0$ to calculate the fraction of slow-decaying radical pairs and the average lifetime of the slow-decaying population.

The results of the fits are collected in Table 1. We can see that for high fields the expected maximum fraction of 50% is indeed achieved. When going to lower fields, the fraction diminishes because the period of the rise time during which the high-field limit is not yet reached is lengthened and part of the population of states $|1\rangle$ and $|4\rangle$ is lost by direct charge recombination through the singlet character still present in these states, resulting in a smaller fraction of radical pairs eventually present in the fully isolated states.

The increase in average lifetime when going to higher fields is explained by the progressively smaller amount of singlet character mixed into the "isolated" levels at long times, after the field has reached its maximum. Due to the large difference in the rates for spin–lattice relaxation and charge recombination at the temperature of the experiment, a small fraction of singlet will have a substantial effect on the average lifetime of levels $|1\rangle$ and $|4\rangle$. We can give a rough estimate for this effect. From

TABLE 1: Fit Parameters Found for the Three-Exponential Fits of the Radical Pair Decay Curves for Times $t > 50$ ms; The Shortest Decay Component τ_1 Was 30−40 ms for All Fits

		lifetimes (s)			fractions[a] (%)		
T (K) (±2 K)	$B_{\max}$ (mT) (±5%)	τ_2 (±5%)	τ_3 (±5%)	$\langle\tau_{\text{slow}}\rangle$[b] (±10%)	$A(\tau_2)$ (±5%)	$A(\tau_3)$ (±5%)	$A(\langle\tau_{\text{slow}}\rangle)$[c] (±10%)
23	6	0.48	0.10	0.19	0.05	0.18	0.24
23	8	0.50	0.12	0.24	0.09	0.19	0.28
23	11	0.90	0.17	0.39	0.09	0.21	0.30
23	15	1.30	0.26	0.54	0.10	0.27	0.37
23	20	1.30	0.26	0.67	0.17	0.26	0.43
23	30	1.68	0.40	0.95	0.21	0.29	0.50
23	40	1.66	0.34	0.90	0.22	0.30	0.52
23	80	~1.66[d]	~0.34[d]	~0.90[d]	~0.22[d]	~0.30[d]	~0.52[d]
23	40	1.66	0.34	0.95	0.22	0.30	0.52
40	40	0.78	0.18	0.34	0.07	0.19	0.26

[a] The exponential functions found were extrapolated to $t = 0$ to calculate fractions. [b] $\langle\tau_{\text{slow}}\rangle$ is the average lifetime of the slow fraction: $(A(\tau_2)\tau_2 + A(\tau_3)\tau_3)/(A(\tau_2) + A(\tau_3))$. [c] The total fraction of slow-decaying radical pairs $((A(\tau_2) + A(\tau_3))/A_{\text{total}})$ at $t = 0$. [d] The fits at 80 mT are unreliable due to the short measuring time caused by excess heating of the magnet (see text). The values printed here are taken from the 40 mT fit because visual inspection showed almost no difference between the traces in the range 40−80 mT.

Table 1 we see that the "pure" lifetime of the isolated levels in high field due to spin−lattice relaxation is at least 1 s and that the charge-recombination lifetime from levels $|2\rangle$ and $|3\rangle$ is about 25 ms.[23] Suppose now that state $|1\rangle$ has an admixture of 1% singlet character. Then the observed rate of recombination from this level will be

$$k_{\text{obs}} = 0.01 \times k_S + 0.99 \times k_R = 0.01 \times 40 + 0.99 \times 1 = 1.39 \quad (8)$$

in which k_{obs} is the observed lifetime of levels $|1\rangle$ and $|4\rangle$, k_S is the singlet lifetime, and k_R is the lifetime of the isolated levels in high field (spin−lattice relaxation time). Thus, the observed rate will be 40% faster than the true high-field lifetime of level $|1\rangle$. This example shows that the true spin−lattice relaxation time might be obscured by the admixture of a quite small fraction of singlet into the isolated levels. We think this strong effect also contributes to the nonexponentiality of the decay curves; the amount of singlet mixed in states $|1\rangle$ and $|4\rangle$ is highly field dependent and so will be the observed lifetime. Since the field is rising exponentially, the average lifetime is a function of time even when $t \gg \tau_{\text{field}}$ because even a small singlet admixture has a drastic effect.

A second reason for the observed field dependence of the average lifetime (Table 1) could be a field-dependent spin−lattice relaxation. Predicting this effect is difficult because this requires detailed knowledge of the phonon distribution, the interaction of the phonons with the external heat bath, etc.[12,30,31] We have attempted to measure the decay at larger fields (40−80 mT), but due to excess heating of the coils, we could monitor the decay curves only for about 1 s. Fits were therefore unreliable; visual inspection showed that the decay curves were virtually identical for fields between 40 and 80 mT (data not shown). This indicates that in this range the spin−lattice relaxation time is not sensitive to the field. We tentatively conclude that the observed change in average lifetime as a function of the magnetic field for fields < 40 mT can be explained by the above described effect of singlet admixture in the isolated levels and that any field dependence of the spin−lattice relaxation for fields < 40 mT is masked by this effect. Because for fields > 40 mT the decay does not change with field, the amount of singlet admixture at long times must then be negligible. Thus the nonexponential behavior for long times observed under these conditions cannot be attributed to time-dependent singlet admixture and must be intrinsic to the decay of fully isolated levels $|1\rangle$ and $|4\rangle$. This suggests that, at least for fields between 40 and 80 mT, spin−lattice relaxation is intrinsically nonexponential. This interesting effect warrants further investigation.

We note that the remaining decrease in absorption at very long times is not an artifact but is caused by a generic (steady-state) magnetic field effect induced by the measuring beam. This (small) effect is presently under study.

The switched magnetic field measurements were done on different preparations of Zn-containing RCs, all yielding similar results. We also performed measurements on Fe-containing RCs. In this case no increase in lifetime was detected when a field was switched on after the flash, supporting our interpretation that the slow component is due to spin−lattice relaxation.

Conclusions

We have shown that populating isolated triplet levels from spin-correlated radical pairs is an effective way to control the lifetime of radical pairs born in the singlet state. Our first results are quite promising; we think the two techniques presented here can be helpful for studying reaction pathways and radical pair products in a variety of donor−acceptor systems.

Both methods presented here are applicable, depending on the available equipment. The switched magnetic field method has the advantage of a relatively simple setup and the possibility of measuring at different field strengths. The main advantage of the pulsed microwave method is its much higher time resolution; additionally its analysis is more straightforward because the eigenfunctions do not depend on time. The maximum fraction of slow-decaying radical pairs achievable in the pulsed microwaves method is < 25% depending on the coupling between the two radicals (eq 7); the pulsed magnetic field method potentially offers a 50% effect when the magnetic field is strong enough and the switching time is short compared to charge recombination.

The switched magnetic field method is in principle an attractive method to measure spin−lattice relaxation as a function of the magnetic field. With our present setup this is as yet not possible, as contamination of the isolated triplet levels with singlet character must be minimized over the field range of interest. Work is in progress to extend the maximum amplitude of the magnetic field well into the high-field limit and to improve the switching time of the field.

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